

'(FILE 'HOME' ENTERED AT 15:12:00 ON 21 MAR 2005)

FILE 'MEDLINE, EMBASE, SCISEARCH, BIOSIS, USPATFULL' ENTERED AT 15:12:20
ON 21 MAR 2005

L1 88251 S (FILTER? OR FILTRAT?) (6P) (CELL? (P) (?BEAD? OR PARTIC? OR ?
L2 9819 S ((TARGET CELL?) OR CELL? (3A) ANTIBOD?) (6P) L1
L3 24677 S (((TARGET CELL?) OR CELL?) (P) (?BEAD? OR ?PARTIC? OR ?SPHERE
L4 2346 S L3 (6P) L1
L5 839 S L4 (6P) L2
L6 826 DUP REM L5 (13 DUPLICATES REMOVED)
L7 826 DUP REM L6 (0 DUPLICATES REMOVED)
L8 256 S L5 (6P) KIT?
L9 256 DUP REM L8 (0 DUPLICATES REMOVED)
L10 13041 S (((TARGET CELL?) OR CELL?) (P) (?BEAD? OR ?PARTICLE? OR ?SPHE
L11 0 S L10 (6P) LL3
L12 13041 S L10 (6P) L3
L13 569 S L12 (6P) L2
L14 189 S L13 (6P) KIT?
L15 189 DUP REM L14 (0 DUPLICATES REMOVED)
L16 61830 S (FILTRAT? OR FILTER?) (3A) (DEVIC? OR APPARAT?)
L17 19 S L16 AND L15
L18 19 DUP REM L17 (0 DUPLICATES REMOVED)

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9 ANSWER 70 OF 71 USPATFULL on STN
ACCESSION NUMBER: 96:3636 USPATFULL
TITLE: Evaluation of transplant acceptance
INVENTOR(S): Buelow, Roland, Palo Alto, CA, United States
PATENT ASSIGNEE(S): Sangstat Medical Corporation, Menlo Park, CA, United States (U.S. corporation)

| | NUMBER | KIND | DATE |
|-----------------------|-------------------------------------|------|--------------|
| PATENT INFORMATION: | US 5482841 | | 19960109 |
| APPLICATION INFO.: | US 1994-247992 | | 19940524 (8) |
| DOCUMENT TYPE: | Utility | | |
| FILE SEGMENT: | Granted | | |
| PRIMARY EXAMINER: | Saunders, David | | |
| LEGAL REPRESENTATIVE: | Rowland, Bertram I., Field, Bret E. | | |
| NUMBER OF CLAIMS: | 13 | | |
| EXEMPLARY CLAIM: | 1 | | |
| LINE COUNT: | 644 | | |

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Alloantigen is extracted from a cellular source, preferably blood cells, with a mild detergent, and partially purified by precipitation of potentially interfering components. The alloantigen preparation is then used in an assay to determine the presence and specificity of receptors specific for alloantigens. The detection of bound receptor is determined by ELISA or other suitable immunoassays.

DETD Especially useful capture agents are **antibodies** against the alloantigen. Instead of whole or intact **antibodies**, one may use **antibody** fragments, e.g., Fab, F(ab').sub.2, light or heavy chain fragments, etc. The use of affinity purified polyclonal **antibodies** or monoclonal **antibodies** is preferred. Immune molecules with alloantigen binding affinity such as CD4, CD8, and T **cell** receptors may also provide useful capture agents, either directly or through derivatives thereof. Lectins may be useful where the alloantigen. . . .

DETD . . . solid or porous and of any convenient shape. Examples of suitable insoluble supports to which the receptor is bound include **beads**, membranes and **microtiter** plates. These are typically made of glass, plastic (e.g. polystyrene), polysaccharides, nylon or nitrocellulose. **Microtiter** plates are especially convenient because a large number of assays can be carried out simultaneously, using small amounts of reagents and samples. Where separations are made by **magnetism**, the support generally includes **paramagnetic** components, preferably surrounded by plastic.

DETD Sample supernatants containing the solubilized alloantigen are then added to separately assayable supports (for example, separate wells of a **microtiter** plate) containing support-bound capture agent. In an example of HLA cross-match, an alloantigen sample from a prospective tissue donor is. . . .

DETD Particular receptors of interest are **antibodies**. The isotypes IgG and IgM will be found in blood, IgA may be detected in secreted fluids, e.g. saliva, etc. Other receptors which may be indicative of an immune response are T-**cell** receptors. Of particular interest are alloantibodies found in the serum of transplant or prospective transplant patients. The volume, composition and. . . .

DETD . . . of non-specifically bound proteins, essentially as described for prior washes. The presence of bound alloantigen-specific receptor is detected with a **labeled** reagent, particularly anti-human **antibodies**, e.g. antisera. Examples of **labels** which permit direct measurement of receptor binding include **radiolabels**, such as .sup.3 H or .sup.125 I, fluorescers, dyes, **beads**, chemiluminescers, colloidal **particles**, and the like. Examples of **labels** which permit indirect measurement of binding include enzymes where the substrate may provide for a colored or fluorescent product. In a preferred embodiment, the **labeled** reagents are **antibodies**, preferably **labeled** with a covalently bound enzyme capable of providing a detectable product signal after addition of suitable substrate. Examples of suitable. . . .

enzymes for use in conjugates include horseradish peroxidase, alkaline phosphatase, malate dehydrogenase and the like. Where not commercially available, such **antibody**-enzyme conjugates are readily produced by techniques known to those skilled in the art.

DETD . . . performed using a reagent which will bind to the bound alloantigen. A convenient positive control for detecting the presence of **antibodies** to HLA Class I antigens is provided by adding to bound alloantigen a known amount of an **antibody** which reacts with human β .sub.2 -microglobulin, an invariant chain found with all HLA Class I proteins. The **antibody** may be directly conjugated to a **label** which allows for detection, or may be used in combination with second **antibody**, particularly the **labeled** detector reagent used in the test samples. The positive control should fall within a pre-determined range, based on what would.

DETD . . . different concentrations from the same source. Thus, one can carry out a plurality of determinations at the same time. Alternatively, **microtiter** plates may be employed where the bottoms of the wells are porous to allow for **filtration**. The particular **device** which is employed will depend upon the number of samples to be determined, available equipment, and the like.

DETD Nunc Maxisorb plates were coated with an anti-HLA class I monoclonal **antibody** (anti-alph 3). The coating solution was 10 μ g/ml of F(ab').sub.2 -TP25 in 0.1M Na Acetate. Each well was coated with. . .

CLM What is claimed is:

12. A **kit** for use in a method for detecting the presence of at least one receptor analyte specific for an HLA antigen in a biological sample, said **kit** comprising: a solid support coated with a capture agent capable of specifically binding to a conserved region of a subset. . .

13. A **kit** according to claim 12, wherein said capture agent is antibody directed to the α 3 domain of HLA Class I heavy. . .

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(FILE 'HOME' ENTERED AT 15:50:08 ON 21 MAR 2005)

FILE 'MEDLINE, EMBASE, SCISEARCH, BIOSIS, USPATFULL' ENTERED AT 15:50:28

ON 21 MAR 2005

L1 405 S ((FILTER? OR FILTRAT?) (3A) (DEVICE? OR APPARAT?)) (6P) ANTIB
L2 169 S L1 (6P) KIT?
L3 1010879 S ANTIBOD? (P) (CELL? OR (TARGET CELL?))
L4 182431 S ?MAGNET? (P) (?BEAD? OR ?PARTICLE? OR ?SPHERE?)
L5 106 S L1 (6P) L3 (6P) L4
L6 93 S L5 AND KIT?
L7 77 S L5 (6P) KIT?
L8 77 DUP REM L7 (0 DUPLICATES REMOVED)
L9 71 S L6 AND (MULTIWELL OR MICROTITER OR MICROWELL)

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